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Review

Signal-Induced Repression: The Exception or the Rule in Developmental Signaling?

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Cell-cell communication plays a key role in organ formation and patterning in multicellular animals and is carried out by a few evolutionarily conserved signaling pathways. The modes of action of these pathways share a number of general properties, or habits, that allow them to strongly activate target genes in a ligand-dependent manner in the proper cellular contexts. Recent studies have revealed that some developmental signaling pathways can also strongly repress genes in a ligand-dependent manner. These new findings raise the interesting possibility that this repressive mode of action is shared by many or most developmental signaling pathways.

Introduction

Patterning and cell specification events during the development of multicellular animals are wrought by a few evolutionary conserved signaling pathways. Signaling via these pathways is used reiteratively throughout development, both in time and space. The signals are interpreted in a tissue- and context-dependent manner, resulting in rapid changes in gene transcription in nuclei of the responding cells. Although the mechanistic details may differ, the pathways share a number of conserved properties or "habits," which include "default repression," "activator insufficiency," and "cooperative activation" (Barolo and Posakony, 2002; reviewed below, and see Figure 1 and Figure 2). The combination of these three habits allows the pathways to robustly activate target genes in response to the signal, in a context-dependent manner, while preventing target gene expression in the absence of the signal.

More recently, it has become clear that the transcription of target genes is not only activated by developmental signals, but that target genes can also be directly repressed. By "directly" we mean that a gene's transcription is repressed, upon signaling, without prior transcriptional induction of a nuclear repressor, i.e., in the absence of de novo protein synthesis. Conceptually this appears to conflict with one of the three habits of developmental signaling pathways, namely that signal-regulated genes are under default repression. If the genes are actively kept off in the absence of the signal, then there would be no opportunity to shut down their transcription upon signaling.

In this review, we first briefly describe the three habits of developmental signaling pathways. Before describing well-studied cases from such signaling pathways, in which genes have been shown to be repressed in a ligand-induced manner, we outline a few theoretical scenarios explaining how signals could actively repress genes. We then describe the defined molecular events leading to gene repression and discuss them in the light of the three habits shared by these signaling pathways. Based on the well-characterized cases of signal-induced repression, we propose possible scenarios of how default repression could be overridden and how active repression could be brought about in other developmental signaling pathways.

The Three Habits of Developmental Signaling Pathways

The Barolo and Posakony review (Barolo and Posakony, 2002) provides a useful framework for thinking about some of the common features of signaling pathways. As described in this review, the development of multicellular animals is controlled by seven major cell-cell signaling pathways (TGFβ, Wnt, Hedgehog [Hh], Notch, receptor tyrosine kinases (RTK), nuclear receptors, and Jak/STAT). Each of these pathways acts repetitively during development, regulating the expression of largely different sets of target genes in distinct tissues and cell types. Although these seven pathways use rather different molecular mechanisms to regulate target genes (see Figure 1, Figure 2, and below), the major consequence of triggering all pathways is the transcriptional activation of specific target genes by signal-regulated transcription factors. These signal-regulated transcription factors bind to specific DNA sequences, called signaling pathway response elements (SPREs), in the promoters or enhancers of the target genes. A pivotal role in signal-induced transcriptional regulation is attributed to these pathway-specific SPREs and the corresponding DNA binding proteins. To achieve a high degree of temporal and spatial specificity of signal responses, the major developmental signaling pathways utilize a number of common habits. These habits have been discussed extensively by Barolo and Posakony (2002) and are only briefly outlined here.

Most developmental signaling pathways appear to regulate gene transcription by a switch mechanism: genes are actively repressed in the absence of the signal and turned on in the presence of the signal. Given the later focus on direct repression, the most relevant habit, for the purposes of this review, is repression of target genes in the absence of ligand, which has been termed default repression. Somewhat surprisingly, default repression in the case of the Wnt, Hh, Notch, and nuclear receptor signaling pathways is exerted on a given gene not only via the same SPRE as signal-induced activation, but also by the same

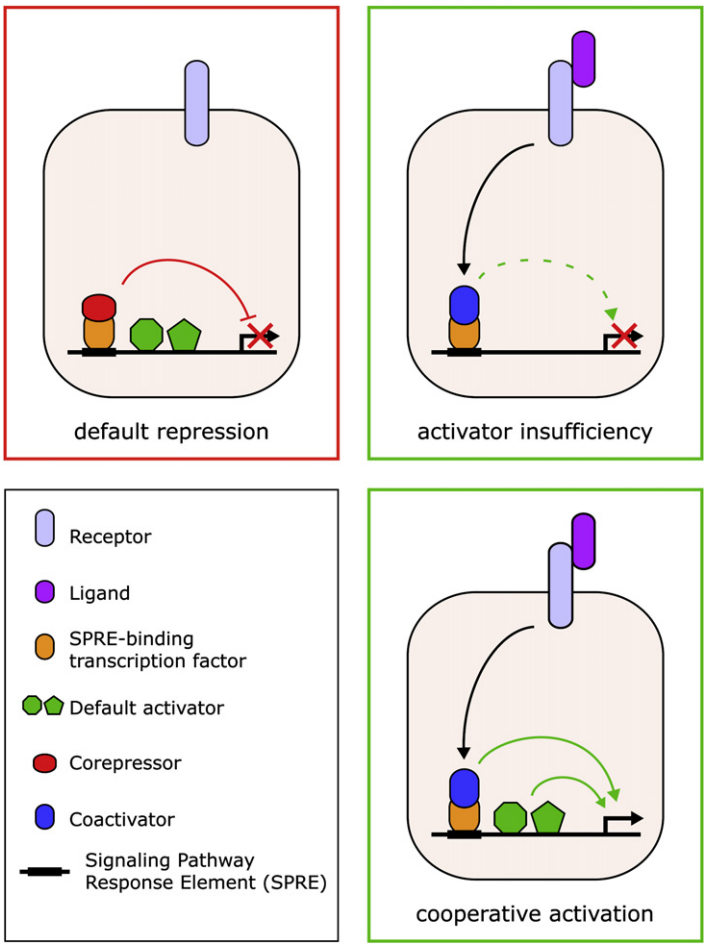

Figure 1. The Three Habits of Developmental Signaling Pathways

Three so-called habits have been proposed to underlie transcriptional activation by all developmental pathways. "Default repression" describes the observation that potential target genes are actively repressed in the absence of signaling events by the binding of repressors to the signaling pathway response elements (SPREs). The binding of signal-modified transcription factors to SPREs, the modification triggered by receptor activation, is not sufficient to fully activate target gene expression, a phenomenon referred to as "activator insufficiency." Only when additional activators and signal mediators are bound do the respective target genes become strongly activated, a process referred to as "cooperative activation." These three habits allow gene expression to be switched from the "off" state in the absence of the signal to the "on" state in the presence of the signal. Figure adapted from Barolo and Posakony (2002).

DNA binding protein, the signal-regulated transcription factors of these pathways. These transcription factors [Lef/T Cell factor (Tcf) in the Wnt pathway, Ci/Gli in the Hh pathway, Su(H)/CBF1 in the Notch pathway, and nuclear receptors themselves] bind to the SPREs in many target genes in the absence of signaling, recruit corepressor complexes (including histone deacetylases [HDACs]), and thus help to keep these target genes repressed prior to signaling (Figure 2). These pathway-specific transcriptional regulators use different mechanisms to become converted from repressors to activators upon signaling (such as protein cleavage, interaction with signal-regulated cofactors, or signal-induced conformational changes). Some of these mechanisms will be outlined below.

The two other habits shared by developmental signaling pathways are referred to as activator insufficiency and cooperative activation. If the binding of signal-activated transcription factors to the corresponding SPRE were sufficient to activate the transcription of target genes, a given signal would activate the same set (and the full set) of target genes in all tissues. However, in different tissue contexts in vivo, the subsets of target genes that are activated overlap only partially or not at all. Indeed, experimental approaches have shown that the simple binding of signal-regulated transcription factors to SPREs does not generally lead to transcriptional activation, a phenomenon referred to as activator insufficiency. Transcription is only significantly increased when these transcription factors join forces with other transcriptional regulators, which often bind in a tissue-specific manner to sequences close to the SPREs. The coordinated action with additional coregulators in signal-induced activation has generally been referred to as cooperative activation. Together with default repression, activator insufficiency and cooperative activation allow genes to be switched from the "off" state in the absence of signals to the "on" state in the presence of the signal in a temporally and spatially coordinated manner (Figure 2; see Barolo and Posakony, 2002, for an extensive discussion of this subject).

Although studies carried out in the last few years have further strengthened the idea of the commonality of the three habits in developmental signaling, it has been shown that certain signaling pathways not only activate gene transcription in a signal-induced manner, but that signaling can also directly repress genes that are transcribed in cells before they respond to the signal. At first sight, signal-induced gene repression conflicts with the principle of default repression. Default repression keeps genes silent in cells prior to signaling via direct binding of default repressors to SPREs. Thus, if genes are kept silent before the signaling events are triggered, there is no apparent need or possibility to repress such genes upon signaling. Indeed, for a signaling pathway to downregulate or repress a previously active gene, default repression of this particular gene would have to be circumvented or overruled, allowing the gene to be transcriptionally active prior to signaling.

From Signal-Induced Gene Activation to Signal-Induced Gene Repression

We will first consider theoretically how the habits outlined above could serve as a roadmap for constructing a signaling system that would allow tissue-specific gene repression (instead of gene activation) upon signaling. The simplest way to obtain signal-induced repression would be to reverse the three habits. Instead of genes being repressed prior to signaling by default repression, genes could be kept active prior to signaling by "default activation." In the simplest case, default activation would be exerted by the same kind of protein species that is used for the signal-induced repression. Upon signaling, the signal-controlled transcription factors would act as repressors. To regulate

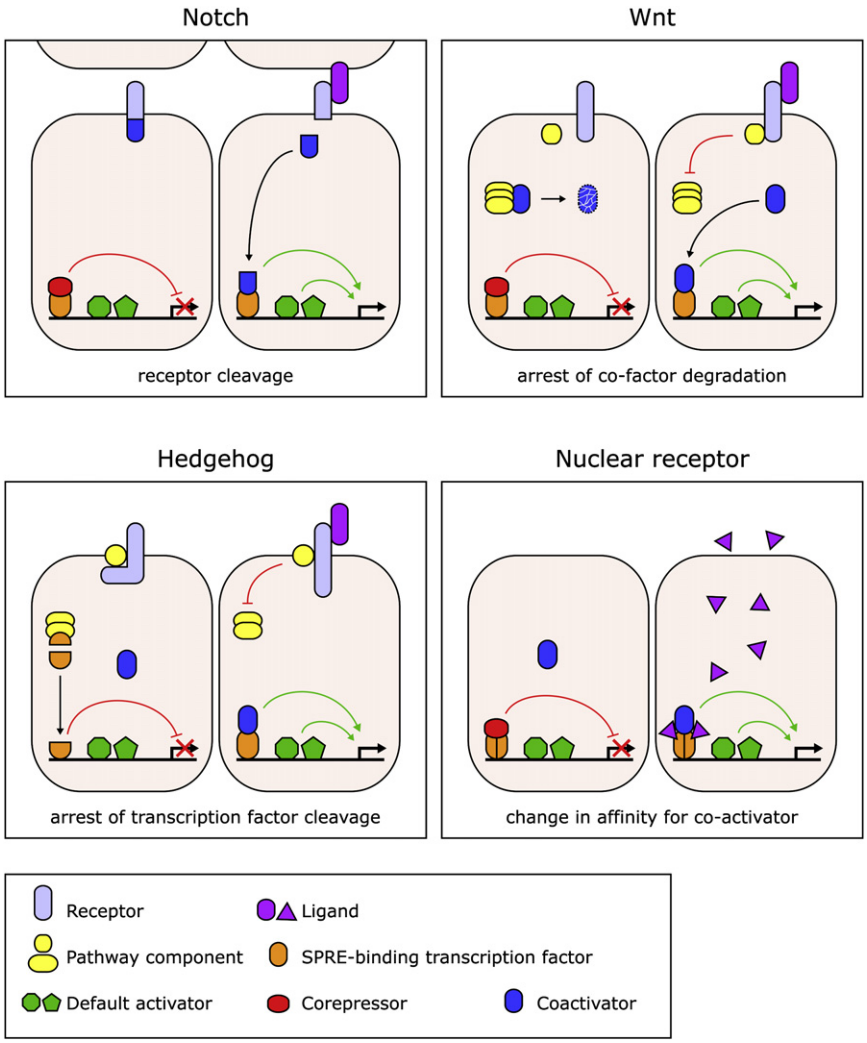

Figure 2. General Outline of Wnt, Hh, Notch, and Nuclear Receptor Signaling

The major developmental signaling pathways (Notch, Wnt, Hedgehog, and nuclear receptor signaling) have evolved different mechanisms that share the same function: switching on target gene expression by changing transcriptional repression to activation following binding of the ligands to their respective receptors. Figure adapted from Barolo and Posakony (2002).

Signal-Induced Repression in the TGFβ Signaling Pathway

The developmental signaling pathway for which the capability to repress target gene transcription in a signal-dependent manner is best understood is the TGFβ pathway (reviewed in Affolter and Basler, 2007; Feng and Derynck, 2005; Massague and Gomis, 2006; Parker et al., 2004; Raftery et al., 2006; Schmierer and Hill, 2007). Signaling is elicited by the binding of TGFβ ligands to two transmembrane receptor serine-threonine kinases, referred to as type I and type II receptors. Upon oligomerization of the ligand-receptor complex, the type II receptors phosphorylate and thereby activate the type I receptors. In turn the latter recruit and phosphorylate receptor-regulated Smads or R-Smads. Subsequently, phosphorylated R-Smads form complexes with a common mediator Smad (Smad4 in vertebrates and Medea in *Drosophila*). These newly formed heteromeric Smad complexes accumulate in the nucleus, where they are directly involved in regulating the transcription of target genes. In agreement with two of the habits of developmental signaling pathways, activator insufficiency and cooperative activation, TGFβ target genes are activated by signaling only when DNA-bound Smad complexes interact with additional transcriptional regulators on native enhancer and/or promoter elements. Smad complexes can interact with a wide variety of other transcription regulators, including DNA binding and non-DNA binding proteins, in order to activate transcription in a signal-dependent manner (Feng and Derynck, 2005; Massague et al., 2005).

While a distinct default repressor has not been identified thus far in TGFβ signaling in vertebrates (see also below), genetic studies have led to the isolation and characterization of a default repressor in the *Drosophila* TGFβ (Decapentaplegic [Dpp]/Bmp) signaling pathway, Brinker (Brk). Most target genes activated by Dpp signaling in different developmental contexts are directly repressed in the absence of signaling by a DNA binding protein encoded by the *brk* gene (Campbell and Tomlinson, 1999; Jazwinska et al., 1999; Minami et al., 1999). Brk recruits corepressors such as Groucho (Gro) and Carboxy-terminal binding proteins (CtBP) to target gene enhancers and represses their activity (Hasson et al., 2001; Winter and Campbell, 2004).

the repression in a tissue-dependent context, these repressors should be insufficient to inhibit transcription by themselves ("repressor insufficiency") but would rather require other transcription factors to mediate repression at specific target sites ("cooperative repression"). Together, these three conceptual habits would allow signaling pathways to strongly repress target genes in a context- and signal-dependent manner, while activating their expression in the absence of the signal.

Since all major developmental signaling pathways directly activate the transcription of many genes upon signaling, repression of target genes must involve ways to circumvent habits in specific cases. Alternatively, some or all of the habits could be context dependent; for example, default repression could be functional only in some cases, releasing certain genes from default repression and making them available for signal-induced repression. Allosteric effects, at the DNA and protein levels, could contribute to such variation, with slightly different binding sites producing opposing effects with regard to transcriptional outcome.

Below we outline in detail the molecular twists allowing several of the developmental signaling pathways to both activate and repress target genes directly in a signal-regulated manner.

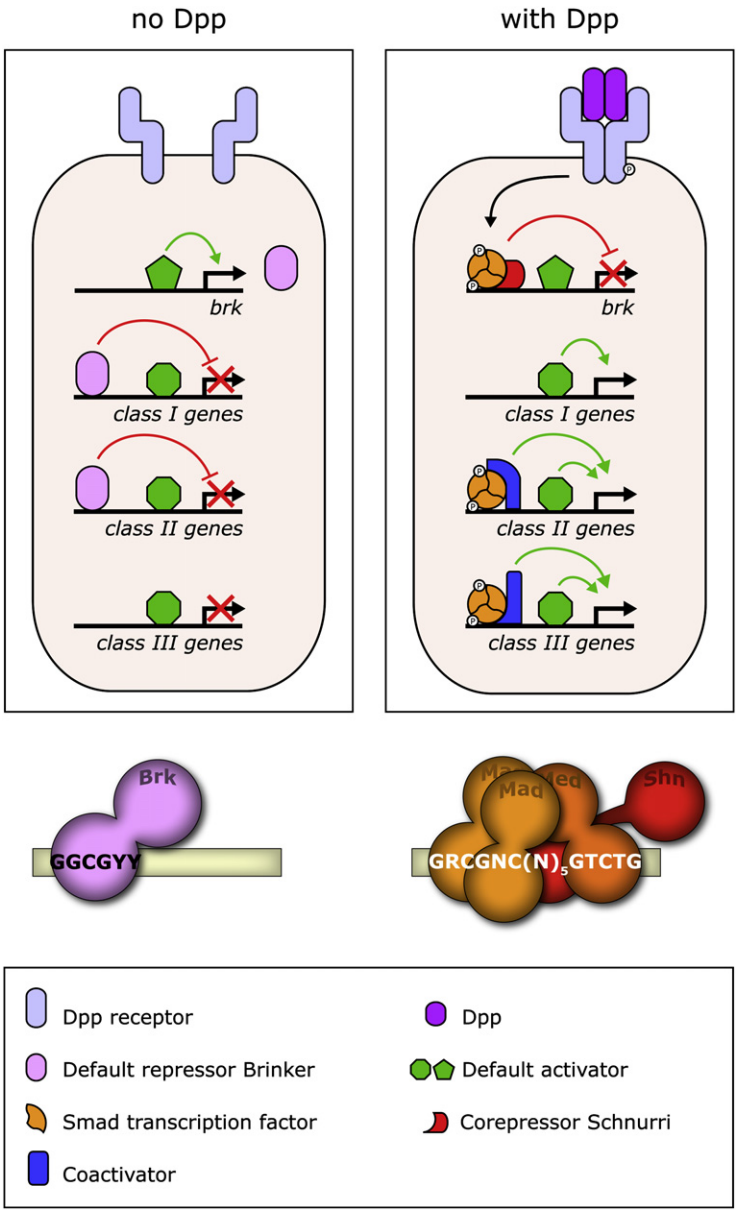

Figure 3. Molecular Aspects of Dpp Signaling in *Drosophila*

In most cells that do not receive Dpp signaling, the *brinker* gene is activated by unknown factors. Brinker is the default repressor of the Dpp pathway and represses its target genes by binding to the consensus sequence GGCGYY (class I and II genes; default repression). Binding of Dpp to its receptors ultimately leads to the formation of an intracellular Smad complex. A trimeric complex of two phosphorylated receptor Smads (R-Smads and Mad) and one Co-Smad (Medea) recruits a corepressor, the large zinc finger protein Schnurri, to well-defined GC-rich binding sequences (GRCGNC(N)5GTCTG) in the *brk* regulatory region, thereby repressing its transcription (signal-dependent, cooperative repression). Repression of *brk* by Dpp leads to the activation of Brinker target gene expression either by Smad-independent activators (class I genes; derepression) or by the interaction of Smads and coactivators with the respective enhancers (class II genes; cooperative activation). A third class of genes is also directly activated by the Dpp pathway via Smad complexes, but this class is not under the repressive control of Brinker.

Therefore, in order for target genes to be transcriptionally activated by Smad complexes, Brk first has to be removed, and this occurs through transcriptional repression of *brk* via Dpp signaling. A combination of genetic and molecular analyses has unraveled a molecular mechanism leading to Dpp-induced transcriptional repression of the *brk* gene. Since these studies represent the most detailed molecular characterization of a signal-induced repression mechanism built into a developmental signaling pathway, our consideration of the Dpp pathway will be further outlined in detail.

The Mechanics of Signal-Induced Repression in the Dpp Signaling Pathway

To understand how activated Dpp signaling leads to the observed transcriptional repression of *brk*, a comprehensive dissection of the *cis*-regulatory region of the *brk* gene was undertaken (Müller et al., 2003). This analysis resulted in the identification of short (16 base pairs) sequence elements referred to as silencer elements (SEs), which are able to produce Dpp-dependent repression when fused to a broadly active enhancer of *brk* or to widely activated regulatory elements of unrelated genes. The SE was shown to bind a complex consisting of two molecules of Mothers Against Dpp (Mad)—the *Drosophila* R-Smad—and one molecule of Medea—the common mediator Smad (Müller et al., 2003; Pyrowolakis et al., 2004; Gao et al., 2005). While the two Mad molecules bind to a GRCGNC sequence motif, Medea binds to a conserved GTCT sequence. At the SE, the Mad/Medea complex recruits the large zinc finger protein Schnurri (Shn); Shn recruitment requires that the distance between the two Smad binding motifs is exactly five nucleotides, and that the fourth nucleotide in the GTCT motif is a T (see Figure 3). Shn is required for the protein complex to repress gene transcription, thus turning the Mad/Medea complex into a transcriptional repressor. Whether the Shn protein recognizes the GTCT motif, or whether the second T in the GTCT motif induces an allosteric change in the Mad/Medea complex that results in the recruitment of Shn, remains to be determined. Further studies have shown that a number of other genes (e.g., *gooseberry*, *bag of marbles*) repressed by Dpp signaling also use SE elements for transcriptional downregulation, and it appears that signal-induced repression via a Mad/Medea/Shn complex is an integral and widely used feature of the Dpp signaling pathway (Pyrowolakis et al., 2004, 2007).

Given the apparent incompatibility of signal-induced repression and default repression, it is interesting to consider how default repression is regulated in the Dpp signaling pathway. Clearly, the Brk protein has to be considered the default repressor of the pathway. Brk binds to the consensus sequence GGCGYY, and such sites have been found in the regulatory regions of most (or all) Dpp target genes. Consistent with the function of these sites in default repression, most or all Dpp target genes can be repressed by ectopic Brk expression in Dpp

responding cells (and cannot be activated in *shn* mutants, which also display ectopic Brk expression); several of these genes also show expanded expression in the absence of *brk*. Many of the Brk sites identified in the fly genome overlap with Mad binding sites (GRCGNC); this means that, somewhat similarly to several of the other developmental signaling pathways (Wingless [Wg]/Wnt, Hh, and Notch pathways, as well as nuclear receptor signaling), the binding sites of the default repressor and the sites to which the signal-induced transcriptional activators bind overlap; however, unlike the case in many of the other signaling pathways, the sequence elements to which the two transcriptional regulators bind are not identical. Importantly, Brk does not bind directly to the Mad/Medea-binding SEs described above (unpublished data); if it did, *brk* transcription would be off in most or all cells of the organism: *brk* would turn off its own expression in the absence of Dpp signaling, and *brk* transcription would be turned off by the Mad/Medea/Shn complex in those cells that experience Dpp signaling.

When all this is taken together, it appears that the default repressor of the Dpp signaling pathway has a unique property—it can bind to many of the Mad sites in the genome (the SPRE of the Dpp pathway), but does not recognize all sites that are recognized by Mad/Medea. This differential binding probably arises from the fact that Brk has a homeodomain-like DNA binding domain (Cordier et al., 2006) that has a very different 3D fold compared with the DNA binding domains present in the signal-induced transcriptional regulators (Mad and Medea; see Shi et al., 1998). Therefore, default repression and signal-induced transcriptional repression are exerted via subtle differences in the sequence elements, allowing the exemption of certain genes from default repression and making them available for signal-induced repression via Mad/Medea/Shn. Consequently, despite the presence of a default repressor in the Dpp pathway, there are a number of genes that carry *cis*-regulatory elements that avoid such repression because of the absence of binding sites for the default repressor. Signal-induced repression is a key event in Dpp signaling and a prerequisite for the subsequent activation of genes upon signaling, because it is responsible for the transcriptional downregulation and thus the removal of the default repressor.

The existence of distinct DNA binding species involved either in default repression (Brk) or in signal-induced activation/repression (Mad/Medea) has also allowed an interesting twist in how Dpp signaling regulates gene transcription. For a number of genes (one of the best-characterized examples being *optomotor blind*), it has been shown that their activation in response to signaling is the result of the removal of repression via Brk; these genes do not require direct binding of Mad/Medea complexes to their *cis*-regulatory regions to become expressed (Jazwinska et al., 1999; Campbell and Tomlinson, 1999; Sivasankaran et al., 2000). The activation of these genes upon signaling can thus be best described as a process of "derepression," leading to the definition of different classes of genes positively regulated by Dpp signaling (class I and class II genes; see Figure 3).

Molecular Scenarios for Signal-Induced Repression in the Vertebrate TGFβ Signaling Pathway

What about activation and repression of target genes via TGFβ signaling in vertebrates? Although the core molecular players, such as the Smad proteins, are the same in vertebrates and in invertebrates, a clear default repressor has not been identified thus far in vertebrates. However, a number of studies have identified genes that are directly repressed by TGFβ signaling, and molecular scenarios accounting for repression have been described.

In epithelial cells, Smad3 associates with E2F4/5, DP1, and p107; this complex moves into the nucleus upon TGFβ signaling and recruitment of Smad4. In the nucleus, the complex recognizes a composite Smad-E2F binding site in the *c-myc* regulatory region and represses *c-myc* transcription (Chen et al., 2002; Frederick et al., 2004). Smad3 can also physically cooperate with ATF3 and repress transcription of the *Id1* gene, which encodes an inhibitor of differentiation in epithelial cells (Kang et al., 2003). In both of these cases, similar to Shn-mediated repression in *Drosophila*, Smad proteins associate with cofactors to bring about repression.

A somewhat different mechanism for signal-induced transcriptional repression has also been uncovered in mammalian cells. The inhibition of osteoblast differentiation by TGFβ is mediated, in part, by the interaction of Smad3 with Runx2, leading to the repression of the transcriptional activity of Runx2 and, therefore, to a repression of the Runx2 target gene *osteocalcin* (Alliston et al., 2001; Kang et al., 2005). In this particular case, the Smad3-mediated repression neither requires the binding of Smad3 to the promoter nor results from decreased binding of Runx2 to its binding site in *osteocalcin*. The repression of Runx2 by Smad3 is mediated by the direct recruitment of class IIa HDACs, specifically HDAC4 and HDAC5, by TGFβ-activated Smad3 to the Runx2 binding sequence in the *osteocalcin* promoter. Similarly, Bmp signaling results in the formation of a complex of Nkx3.2, HDAC1, and Smad1, leading to inhibition of the transcriptional activity of Nkx3.2 (Kim and Lassar, 2003). Although Smads might not bind directly to the target sites via Smad binding DNA elements in these two cases, the activated Smad complexes are recruited upon signaling to the promoters via interaction with other transcription factors, resulting in signal-induced transcriptional repression.

A number of studies have demonstrated that activated Smad proteins can repress target genes even more indirectly by sequestering transcriptional activators in a form that does not allow them to bind to their target sites, leading to transcriptional downregulation. In response to TGFβ, Smad3 has been shown to suppress the transcriptional activation potential of MyoD and Myogenin through direct interaction with the HLH domains of the two proteins (Liu et al., 2004). Although this mechanism of signal-induced repression (or signal-induced deactivation) is somewhat more indirect, because it does not require direct DNA binding of the Smad complexes to target genes, it is as effective in inducing transcriptional repression as the other scenarios: it is dependent on the activation of the TGFβ signaling pathway, and it does not rely on the prior transcriptional activation of a repressor that then acts on target genes. As illustrated below, this mechanism of repression (deactivation) is widely used by hormone receptors. In literature dealing with hormone receptors the mechanism is referred to as "transrepression," and we will use this term to describe this particular mode of action (see also Figure 4).

The above examples from vertebrate and invertebrate systems demonstrate that signal-induced repression is a reoccurring

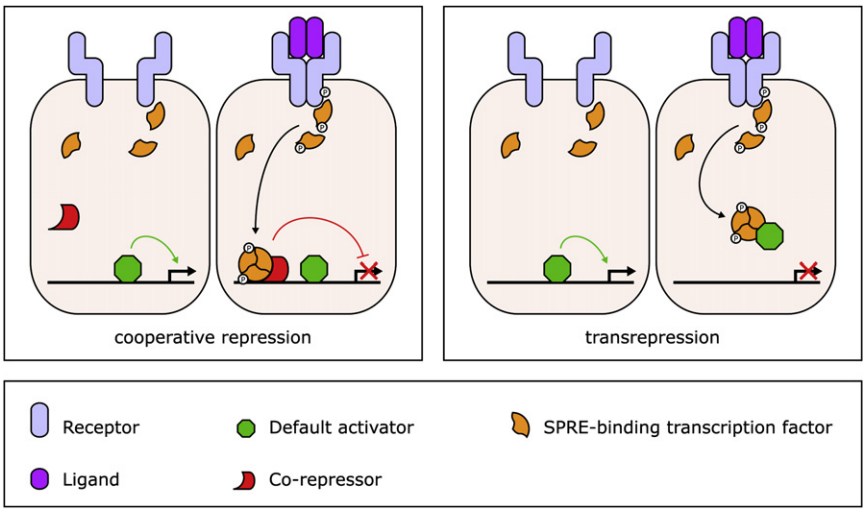

Figure 4. Molecular Mechanisms Involved in Signal-Induced Repression in TGFβ Signaling

Similar to the habits proposed for switching from transcriptional repression to activation in response to a developmental signal, some general principles may emerge from transcriptional repression in the TGFβ pathway. The corepressor (Shn) is recruited to the SPRE upon activation of the signaling pathway by the Smad complexes and is required to efficiently repress genes ("cooperative repression"). However, repressive mechanisms are not limited to direct DNA binding of signal mediators. The sequestration of activating transcription factors (for example in the cytoplasm) by members of the Smad complexes can also abrogate gene activation ("transrepression"). This mechanism exemplifies how genes without SPREs can be directly controlled by the TGFβ signaling pathway.

theme and an important feature of the cellular response to signaling via members of the TGFβ superfamily of signaling molecules. Distinct molecular complexes (Mad/Medea/Shn sites not recognized by the default repressor Brk, Smad proteins bringing associated repressor proteins to DNA-bound activators, and Smad proteins sequestering transcriptional activators) uncouple certain genes from the effects of default repression, allowing them to be actively transcribed in cells in the absence of the signal (default activation) and to be transcriptionally repressed ("co-operative repression") by signal-induced Smad complexes (Figure 4).

Since signal-induced, active repression is so widespread in the cellular response to TGFβ signaling, and is achieved by various means, it would come as a surprise if repression were not an important feature of other developmental signaling pathways, especially if we consider the conservation of other mechanistic themes. We will now turn to the Wnt signaling pathway, and summarize what is known about default repression and signal-induced repression in Wnt signaling.

Signal-Induced Repression in the Wnt Pathway

Signaling proteins of the Wg/Wnt family are secreted glycoproteins that control diverse processes in tissue development and homeostasis (Clevers, 2006; Logan and Nusse, 2004). In the absence of Wg/Wnt signals, DNA-bound transcription factors of the Tcf family of HMG-box proteins bind the transcriptional corepressors Gro/TLE and CtBP, thereby repressing Wg/Wnt target genes via default repression (Cavallo et al., 1998; Hurlstone and Clevers, 2002). Activation of the canonical Wnt/β-catenin pathway stabilizes the β-catenin protein, allowing its relocation from the cytoplasm to the nucleus. In the nucleus, β-catenin heterodimerizes with Tcf, displacing TLE and HDAC corepressor complexes, thereby converting the default repressor Tcf into an activator complex by recruiting coactivators such as CREB binding protein (CBP)/p300 and Brahma-related gene-1 (Brg1) for chromatin remodeling (reviewed in Parker et al., 2007; Städeli et al., 2006; Willert and Jones, 2006; see also Figure 2). The coactivator function of β-catenin depends mainly on two domains of the protein, an N-terminal part that has been shown to interact with Legless/B cell lymphoma 9 (BCL9) and thereby recruit Pygopus (Belenkaya et al., 2002; Kramps et al., 2002; Parker et al., 2002; Thompson et al., 2002), and a C-terminal part binding to TATA binding protein (Hecht et al., 1999), Brahma/Brg1 (Barker et al., 2001), CBP/p300 (Hecht et al., 2000; Takemaru and Moon, 2000), Mediator subunit 12 (MED12) (Kim et al., 2006), and Hyrax/Parafibromin (Mosimann et al., 2006). Recently, more β-catenin binding proteins have been identified and shown, in addition to contributing to activation, to regulate the affinity of β-catenin for Tcfs (Li and Wang, 2008; Tran et al., 2008; see also Parker et al., 2007). The exact mechanisms by which β-catenin employs these cofactors to control the switch of Lef/Tcf from default repression to signal-induced cooperative activation remain poorly understood. Their elucidation represents a field of intense research activities, notably due to the immense interest in blocking Wnt signaling in certain cancers.

Over the past few years, members of the Tcf family of transcription factors have been the only unambiguous DNA binding partners accounting for the diverse β-catenin-dependent nuclear transcription programs (Arce et al., 2006; Hurlstone and Clevers, 2002). In *Drosophila*, the Tcf homolog Pangolin has been shown to follow the three habits of developmental signaling pathways, and to be essential for both default repression of a number of target genes in the absence of Wg and cooperative activation in its presence (Brantjes et al., 2002; Brunner et al., 1997; Lawrence et al., 2000; Schweizer et al., 2003; van de Wetering et al., 1997). In vertebrates, four genes encode Tcf family transcription factors: *TCF1*, *TCF2*, *TCF4*, and *LEF1*. Some of these factors are more dedicated to β-catenin-dependent repression of target genes in the absence of the signal (Tcf3), and some more dedicated to β-catenin-dependent activation of Wnt target genes (Tcf4 and Lef1); Tcf1 appears to have the ability to do both (Arce et al., 2006; Hoppler and Kavanagh, 2007; Houston et al., 2002; Roose et al., 1999; Shitashige et al., 2008).

Despite the widespread notion that Wg target genes are activated by Wg/Wnt ligands, the direct, signal-induced repression of a considerable number of genes has been reported (see http://www.stanford.edu/%7ernusse/wntwindow.html for details). However, in only very few of these cases have detailed molecular studies addressed the *cis*- and *trans*-acting factors involved in the transcriptional repression.

In three cases, evidence has been presented implicating Lef/Tcf binding sites in signal-induced repression of target genes: in the regulation of E-cadherin transcription in murine hair follicle morphogenesis, in the regulation of *stripe* expression in the *Drosophila* embryonic epidermis, and in the control of *dpp* expression in the leg imaginal disc (Jamora et al., 2003; Parker et al., 2008; Piepenburg et al., 2000; Theisen et al., 2007). In all these cases deletion of Tcf binding sites led to a failure to repress transcription in a signal-dependent manner. How such binding sites assemble protein complexes to repress transcription in a Wnt-dependent manner remains mysterious, and it is unclear how these target genes escape default repression.

Several studies have provided insight into the role of other transcription factors as DNA binding partners for β-catenin, targeting Wnt-dependent activation, as well as repression, to genes that lack Lef/Tcf binding sites and instead carry sequences recognized by the DNA binding domains of these additional β-catenin binding partners. Analogous to its role as a binding partner for Tcf, β-catenin can interact with Pitx2, converting it from a transcriptional repressor to a transcriptional activator (Kioussi et al., 2002). During development of the anterior pituitary it was shown that Prop1 can also serve as a β-catenin cofactor, activating the expression of another critical lineage-determining factor, *Pit1* (Olson et al., 2006). Strikingly, in the same cell lineage, Prop1/β-catenin complexes also function as Wnt-signaling-dependent transcriptional repressor complexes, which is important in mediating cell lineage determination due to the promoter-specific repression of *HesX1* (Olson et al., 2006). In all of these cases, β-catenin interacts in the nucleus with DNA binding proteins other than Tcf. In the later case, where this interaction results in repression, Tcf-dependent default repression has been elegantly circumvented, because the repressed Wnt target gene in question (*HesX1*) does not harbor Tcf binding sites (but rather binding sites for the other β-catenin binding partner Prop1).

A very recent study has unraveled a novel and rather surprising mechanism by which Wg signaling can lead to the direct repression of *Drosophila* target genes (Blauwkamp et al., 2008). Cadigan and colleagues have identified and characterized a number of genes repressed by Wg signaling in cultured cells. They find that the repression of three out of four genes analyzed did indeed require both β-catenin and dTCF. However, detailed analysis of the *cis*-regulatory region of one of these genes, *Ugt36Bc*, revealed that the β-catenin/Tcf complex recognized DNA sites (AGAWAW) that were markedly different from typical Tcf binding sites (CCTTTGATCTT), suggesting that, at such sites, allosteric effects (and possibly neighboring DNA binding factors) convert the signal-induced Tcf complex from an activator to a repressor complex. In addition, the novel Tcf binding sites were not only required for Wg-induced repression, but were also essential for the transcription of *Ugt36Bc* in the absence of signaling. These studies suggest that distinct Tcf binding sites can change the activity of the associated Tcf complex, both in the presence and in the absence of signaling. Interestingly, Blauwkamp et al. (2008) also show that a mutant form of β-catenin defective in activation is still functional for repression, indicating that distinct domains of the protein are required for each activity. This result suggests that allosteric effects, in part, may influence the availability of distinct domains of β-catenin for interactions with transcriptional coregulators. It will be interesting to find out whether genes in the *Drosophila* genome other than *Ugt36Bc* are also regulated by this novel mode of action of Tcf/β-catenin.

Thus, several paradigms have emerged that can lead to Wg/Wnt-induced repression, such as the use of an alternative DNA binding partner for β-catenin and the use of alternative DNA binding sites for Tcf (Figure 5). A detailed molecular characterization of several cases in which typical Tcf binding sites have been associated with signal-induced repression (i.e., *E-cadherin*, *stripe*, and *dpp*) might uncover additional mechanisms involved in active, signal-induced repression.

Signal-Induced Repression in the Nuclear Receptor Signaling Pathway

Lipophilic ligands exert their physiological effects through transcriptional control of target genes via nuclear receptors. In most of the cases analyzed so far, nuclear receptors act as ligand-inducible, DNA binding transcriptional activators. Many of these receptors are bound to DNA also in the absence of the ligand, and act as default repressors. Thus, it looks like the transcription-activating function of nuclear receptors can be well explained using the three habits proposed by Barolo and Posakony (2002). The switch from repression to activation upon ligand binding has been studied extensively using biochemical approaches in cultured cells and has provided insight into the molecular mechanisms underlying the combinatorial role of the multiple cofactor complexes that are required for mediating transcriptional activation or repression. These studies make nuclear receptor signaling the best-studied developmental signaling system with respect to the basic molecular processes involved in transcriptional control (Aranda and Pascual, 2001; Gronemeyer et al., 2004; Perissi and Rosenfeld, 2005; Rosenfeld et al., 2006).

In addition to activating genes upon ligand-receptor interaction, a number of nuclear receptors have been shown to repress gene transcription in a ligand-induced manner. For some of these nuclear receptors, such as the thyroid hormone receptor (TR), the specific spacing and orientation of subelements of core binding sites (SPREs) is critical in determining either positive or negative gene regulation in response to ligand. At so-called negative thyroid response elements (nTREs), TR activates expression in the absence of ligands, and this activation is blocked by T3 treatment (Lazar, 2003; Nygard et al., 2006; Nygard et al., 2003; Saatcioglu et al., 1993). These studies point to allosteric effects of DNA binding sites dictating coregulator interactions (see Rosenfeld et al., 2006 for an extensive discussion of this topic).

In several other cases, the transcriptional stimulation of well-known activator proteins can be suppressed by active, ligand-bound nuclear receptor complexes, a phenomenon referred to as transrepression. An example is the negative regulation of inflammatory responses by nuclear receptors, which has been intensely studied due to the key role of inflammation in both immunity and pathogenesis of chronic diseases, such as arteriosclerosis, diabetes, and neurodegenerative diseases (reviewed in Rosenfeld et al., 2006). Several different molecular scenarios leading to the transrepression of NFκB- and AP-1-activated genes have been uncovered, and many of these events do not require sequence-specific DNA binding of nuclear receptors. Transrepression can block gene expression by affecting the

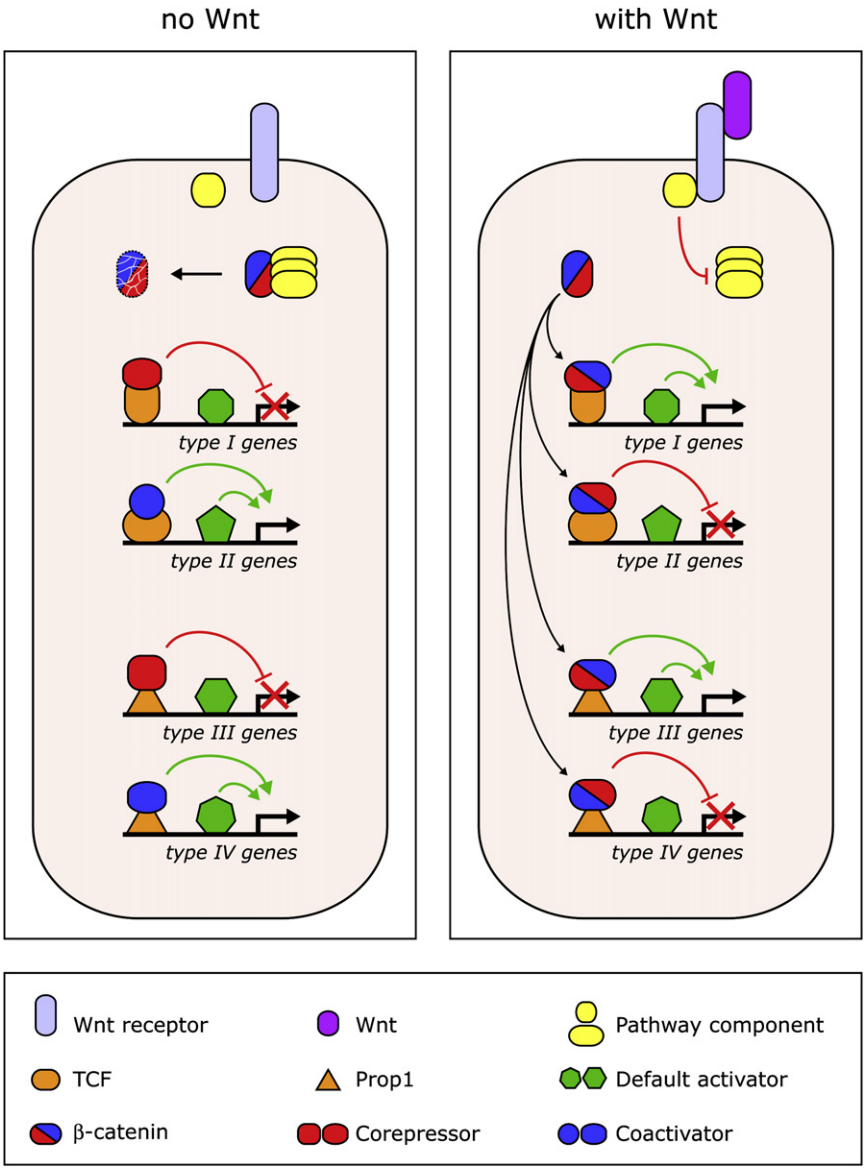

Figure 5. Molecular Mechanisms Involved in Signal-Induced Repression in Wg/Wnt Signaling

Ligand-induced activation of the Wnt pathway leads to either transcriptional activation or repression, depending on the respective *cis*-regulatory elements (SPREs) or their sequence environment. In cells that do not receive a Wnt signal, Tcf target genes are either repressed by recruitment of Tcf and corepressors to the canonical Tcf sites with the general consensus CCTTTGATCTT (type I genes) or activated by recruitment of Tcf and coactivators to AGAWAW sites (type II genes). Following Wnt stimulation, the continuous degradation of β-catenin is inhibited; β-catenin binds to Tcf and displaces the prebound transcriptional coregulators. Due to the different properties of the two distinct Tcf/β-catenin complexes, type I genes are activated whereas type II genes are repressed upon Wnt signaling. In a few specific cases, β-catenin has been shown to act via a non-Tcf transcription factor. The interaction of β-catenin with DNA-bound Prop1 can lead to either gene activation (type III) or repression (type IV), depending on the context of the *cis*-regulatory elements. Remarkably, this scenario allows genes without Tcf binding sites to be directly controlled by Wnt signaling.

recruitment of coactivators to NFκB (nuclear receptors block coactivator recruitment, thus repressing NFκB-mediated transactivation), or by allosterically controlling the coregulator conformation (nuclear receptors interact with coregulators of NFκB and change their function from a coactivator to a corepressor). In most of these cases, signal-induced transrepression has been demonstrated to be selective for specific subsets of genes that are activated by a particular activating signal, implying an additional requirement for context- and/or promoter-specific mechanisms (Luecke and Yamamoto, 2005; Ogawa et al., 2005; see Rosenfeld et al., 2006). Because in most of these cases transrepression does not require the DNA binding of nuclear receptors, default repression is not an issue.

Signal-Induced Repression in the Other Developmental Signaling Pathways

While signal-induced repression has been well studied in TGFβ signaling, and represents an emerging theme in Wnt and nuclear receptor signaling, much less is known about repression in Hh, Notch, RTK, and Jak/STAT signaling. Most schematic representations in review articles dealing with the Hh and the Notch signaling pathways depict these as a switch, converting a DNA-bound default repressor complex into a DNA-bound activator complex upon signaling (Bray, 2006; Huangfu and Anderson, 2006; Hurlbut et al., 2007; Ruiz i Altaba et al., 2007). The DNA binding domains of the default repressors [Ci/Gli in the Hh pathway, and Su(H)/CBF1 in the Notch pathway] are identical to the DNA binding domains of the activators, and they thus regulate default repression and cooperative activation through the same SPREs. As outlined above, there is a certain conflict of habits when considering signal-induced repression, unless certain conditions are met. To our knowledge, a molecular scenario leading to signal-induced repression has not been described for the Hh and Notch pathways, or for Jak/STAT signaling. Surprisingly, although RTK signaling has been studied quite intensively, we are not aware of a bona fide example of a gene directly repressed upon RTK signaling. This is unexpected considering that default repression and signal-induced activation are in some cases achieved by different DNA binding transcription factors. Yan/Aop, an Ets domain transcriptional repressor, is critically involved in many different RTK signaling processes and can be considered a default repressor of the pathway. Activation of the Ras/MAPK pathway involves phosphorylation, nuclear export, and subsequent degradation of Yan/Aop. At the same time, Ras/MAPK signaling phosphorylates another Ets factor, Pointed (Pnt), that acts as a transcriptional activator to positively regulate target genes.

This mode of transcriptional switch involving the two antagonistic Ets proteins Yan and Pnt is probably a general one; the two proteins seem to be differentially regulated by RTK signals and to antagonize each other in a variety of contexts during *Drosophila* development, for example in the developing *Drosophila* eye (Tootle and Rebay, 2005), during dorsal mesoderm cell fate specification (Halfon et al., 2000) or during D-V axis formation (Gabay et al., 1996). Moreover, data from vertebrates point to the evolutionary conservation of this mechanism; a homolog of Yan, the Ets factor TEL1/ETV6, exists in mammals and acts as a transcriptional repressor; the activity of another Ets factor, the tumor suppressor ERF, is regulated by Ras/MAPK signaling similarly to Yan/Aop. In the absence of RTK signaling, ERF binds directly to Ets binding sites of target genes, including *myc*, and represses their transcription. Upon signal induction ERF is phosphorylated and exported from the nucleus to allow expression of target genes.

Interestingly, another example of default repression in the RTK signaling pathway involves a protein that is structurally unrelated to the abovementioned Ets domain proteins. Capicua (Cic), an evolutionary conserved HMG domain transcriptional repressor, is widely involved in controlling the nuclear readout of RTK signaling; its role has been best established in *Drosophila* (Furriols and Casanova, 2003). RTK signaling mediated by the Torso receptor defines the terminal regions of the early *Drosophila* embryo by locally derepressing the gap genes *tailless* and *huckebein* (Cinnamon et al., 2004; de las Heras and Casanova, 2006; Jimenez et al., 2000). In this case, and also later in the activation of *zerknüllt* in the same regions of the embryo, Torso signaling activity seems centered on the posttranscriptional downregulation of Cic; signaling removes the default repressor, thereby releasing target genes from transcriptional repression. Recently, EGF-dependent removal of Cic was also shown to be important for proper cell proliferation in larval tissues. Again, the role of RTK signaling seems to be restricted to the removal of the default repressor (Tseng et al., 2007). These results suggest that the RTK pathway uses at least two distinct default repressors. In the case of the Ets domain proteins of the Yan/Aop class, the same DNA binding site is also used for signal-induced transcriptional activation. In the case of Cic, however, target genes are not activated by a switch mechanism, but solely by relief of repression; activated Ets proteins do not seem to be involved. Yet despite the existence of two distinct mechanisms bringing about default repression, a molecular species bringing about active, signal-induced repression has not been described so far in RTK signaling.

General Considerations

Developmental signaling pathways regulate gene transcription to a large extent by a switch mechanism; genes are actively repressed in the absence of the signal, and actively turned on in the presence of the ligand. This switch involves the replacement of corepressor complexes with coactivator complexes, ultimately resulting in effective transcription of the signal-regulated genes via RNA polymerase II. In recent years, it has become increasingly evident that some of the developmental signaling pathways can also efficiently repress genes directly in a ligand-induced manner. The question therefore is how signal-dependent repression-to-activation switch mechanisms are compatible with a "reverse switch," in which the transcriptional activity of a target gene is high in the absence of the signal and low in its presence.

For several of the developmental signaling pathways, molecular scenarios accounting for the observed signal-induced repression have been elucidated. In Dpp signaling, specific sequence elements called SEs have been identified; such SEs recruit the Smad signal mediators together with the large zinc finger protein Shn, which acts as a corepressor (or recruits corepressor complexes). Shn recruitment only occurs on elements in which the Smad binding sites are appropriately spaced, and which conform to a rather strict consensus sequence. Genes (or enhancer elements) repressed through such SEs are not under the control of default repression since most of them do not harbor binding sites for the default repressor Brk. Brk is a DNA binding protein with DNA binding properties slightly different from those of the Smad transducers.

Although the role of Shn in vertebrates is much less clear (Shn might act as a coactivator in vertebrates; see Yao et al., 2006), TGFβ signaling in vertebrates also triggers transcriptional repression. Smad protein complexes can interact with a number of different proteins and bring corepressor complexes to their DNA binding sites, resulting in signal-regulated repression of target genes. In a somewhat more indirect way, activated Smad complexes can transrepress the activation potential of certain transcription factors (such as MyoD), leading to effective repression of MyoD-dependent target genes. Transrepression also represents a prominent pathway for nuclear receptors to repress target genes in a signal-induced manner. Since transrepression in many, if not most, cases does not involve direct DNA binding of signal mediators, genes under transrepressional control are not targeted by default repression of the signaling pathway, because they lack the corresponding SPREs.

Interestingly, evidence is emerging that both the Wg/Wnt pathway and the nuclear receptor pathway can work in situations resembling a simple reversal of the three habits. Certain genes are turned on in the absence of a signal by Tcf or nuclear receptors through the recruitment of coactivator complexes; upon signaling, genes are turned off by the same DNA binding proteins following an exchange of coactivator for corepressor complexes. Since it appears that the sequences of SPREs operating under the regime of the standard versus reverse habits differ somewhat, it is likely that conformational changes influence the transcriptional outcome by modulating the nature of DNA-protein complexes that assemble.

Strong evidence that transcription factors can change their properties from activators to repressors depending on the sequence they bind to has been obtained some time ago. For example, it has been shown that Pit 1 and NFκB, depending on the target sequence they bind to, can act either as activators or as repressors (Leung et al., 2004; Scully et al., 2000). From these and many other studies, the paradigm has emerged that specific transcription factors can use distinct combinations of cofactors, depending on cell type, promoter, DNA binding site, or the action of various signaling pathways/ligands. Apparently, this flexibility in the mode of action of transcription factors also holds true, to some extent, for developmental signaling mediators, such as Smads, Tcf/β-catenin, and nuclear receptors. It will be interesting to find out whether such flexibility in output is also a property of other signal-mediating transcription factors,

such as Ci, Ets domain proteins implicated in RTK signaling, or Su(H)/CBF1, and might thereby account for signal-mediated repression in these pathways.

How important is the role of signal-induced repression in developmental signaling? Microarray studies show that the expression of a large number of genes changes in response to pathway activation: while for many genes expression increases, for an equal number expression is repressed. For both classes, induced and repressed, a distinction between direct and indirect action of the involved signaling mediators has to be made. This has been done for individual genes, but we lack a more global view because such analyses are tedious and require a detailed examination of the *cis*-regulatory elements and the transacting factors interacting with them. An involvement of transrepression for those genes that are repressed in a signal-mediated fashion but lack recognizable SPREs can be neither positively nor negatively inferred without extensive biochemical analyses. This might be the reason for the dearth of evidence for transrepression in *Drosophila*. Due to these limitations, it will take quite some time to properly determine the importance and true contribution of signal-mediated repression to developmental signaling control.

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